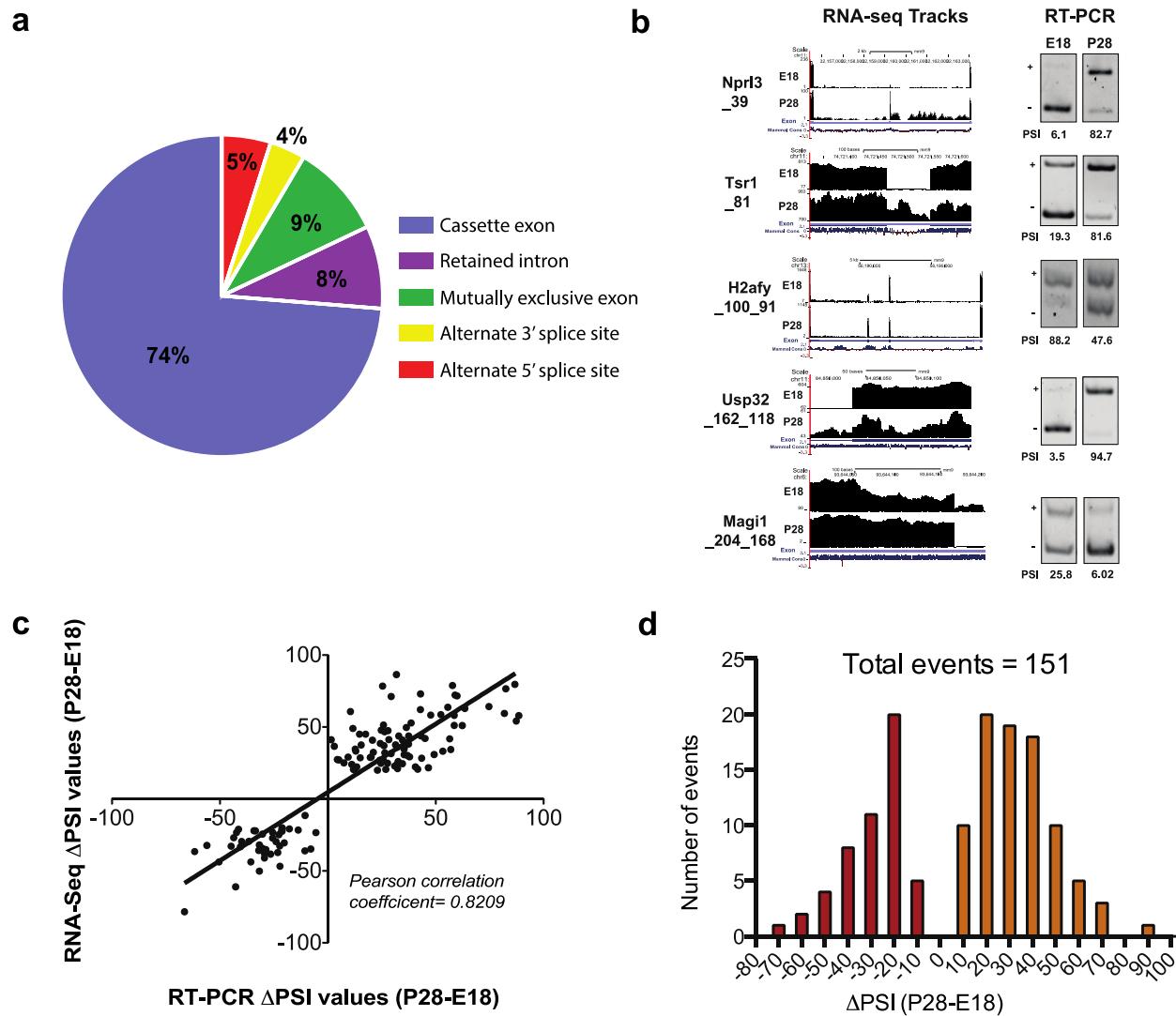


Supplementary Information

Supplementary Figure 1.



Supplementary Figure 1. Validation of alternative mRNA splicing during mouse liver development

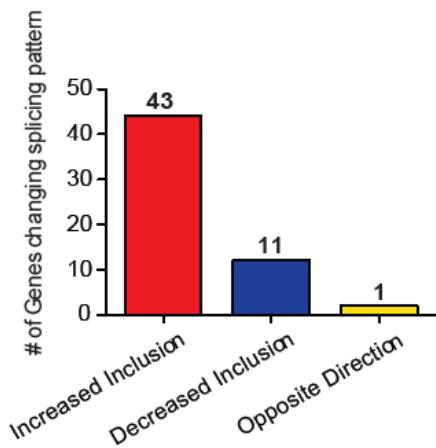
(a) Pie chart of the different types of alternative splicing (AS) events analyzed in this study. **(b)** Left panel shows RNA-seq data displayed on the UCSC genome browser for each event. Right panel shows representative gel images of RT-PCR validation of the same AS events. The bands corresponding to (+) indicate exon inclusion and (-) indicates exon exclusion. E18 corresponds to embryonic day 18 and P28 corresponds to postnatal day 28 liver samples. **(c)** Scatter plot showing comparison of RT-PCR and RNA-seq based ΔPSI (Difference in Percent Spliced In) values for 179 events. **(d)** Frequency Distribution table of exon inclusion and skipping events based on the ΔPSI (P28-E18) values.

Supplementary Figure 2.

a

Regulation Pattern	% Sequence Similarity	Variable Region in frame
Mouse (>20%), Human (>10%)	84	40/55
Mouse (>20%), Human (<10%)	90	28/33
Mouse (>20%), Human single isoform	78	26/39

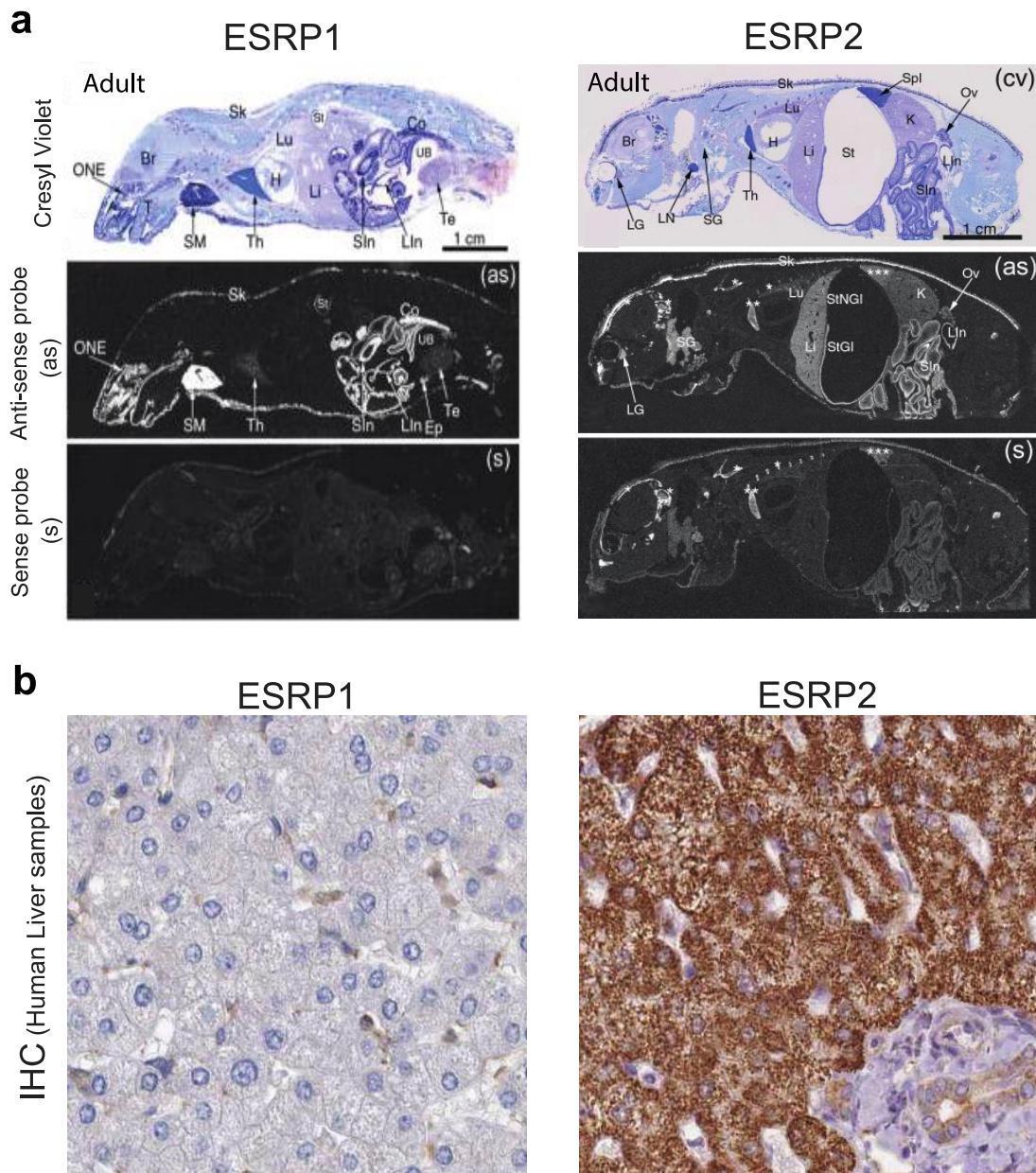
b



Supplementary Figure 2. Conservation of splicing in mouse and humans during liver development

(a) The table represents average sequence similarity for each of the three splicing categories and the number of exons that maintain the original frame of translation in mouse and human AS during liver development. (b) Direction of splicing for the 55 events that showed regulation in both mouse and human.

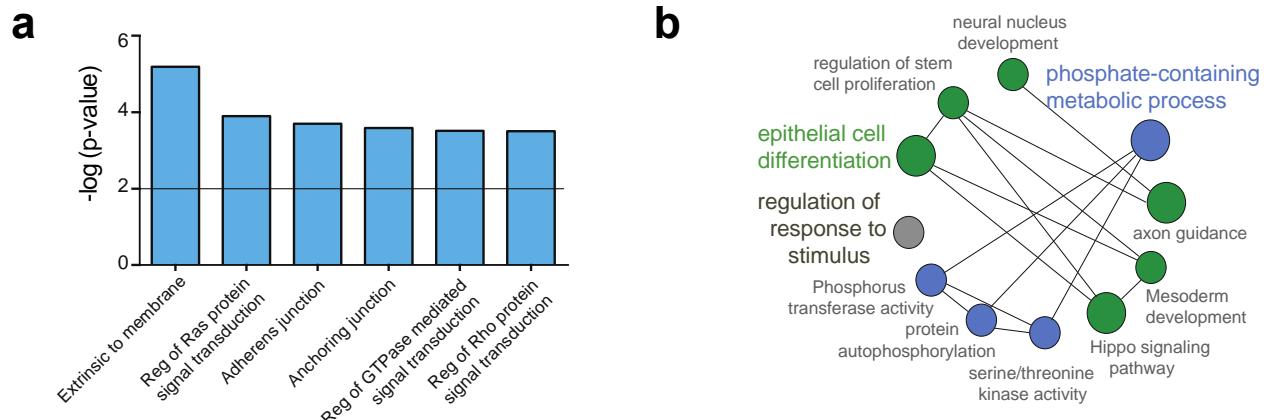
Supplementary Figure 3.



Supplementary Figure 3. Expression of ESRP1 and ESRP2 in various tissues

(a) In-situ hybridization analysis demonstrating the expression of ESRP1 (left panel; obtained from Warcheza et al. 2009) and ESRP2 (right panel) in various mouse tissues by X-ray film autoradiography detection of ESRP1 and ESRP2 mRNAs. Abbreviations: Br –brain; Co – colon; Ep – epididymis; H – heart; K – Kidney; LG- Lacrimal Gland; Li – liver; LIn – large intestine; LN- Lymph node; Lu – lung; ONE –olfactory neuroepithelium; Ov - ovaries; SG- salivary Gland; Sk – skin; SIn – small intestine; SM – submaxillary gland; Spl – spleen; St – stomach; T – tongue; Te – testis; Th –thymus; UB – urinary bladder. **(b)** IHC staining of ESRP1 and ESRP2 in human liver tissue images obtained from The Human Protein Atlas (www.proteinatlas.org¹).

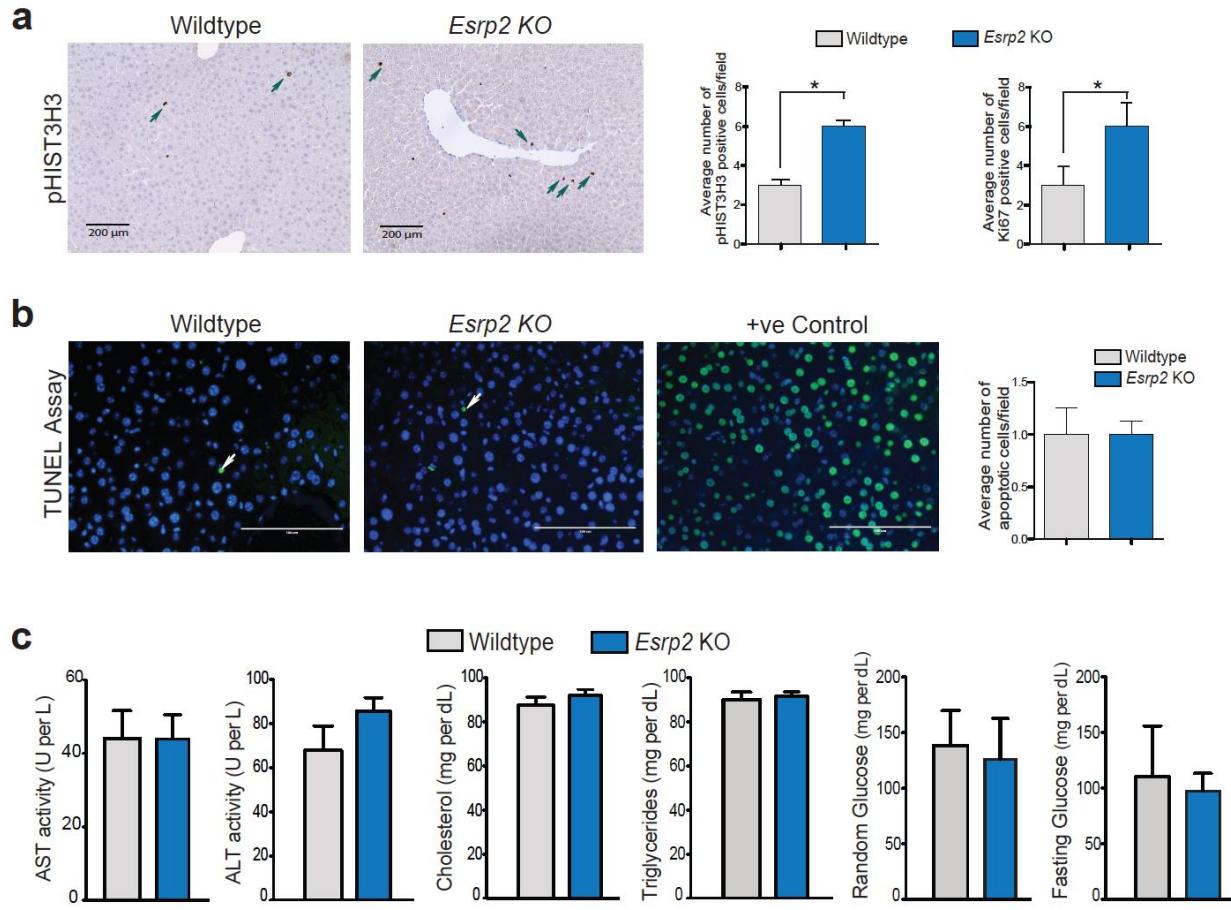
Supplementary Figure 4.



Supplementary Figure 4. Analysis of the ESRP2 regulated splicing network in the liver

(a) Gene Ontology analysis of ESRP2 target genes. **(b)** Protein-protein interaction analysis of ESRP2 target genes.

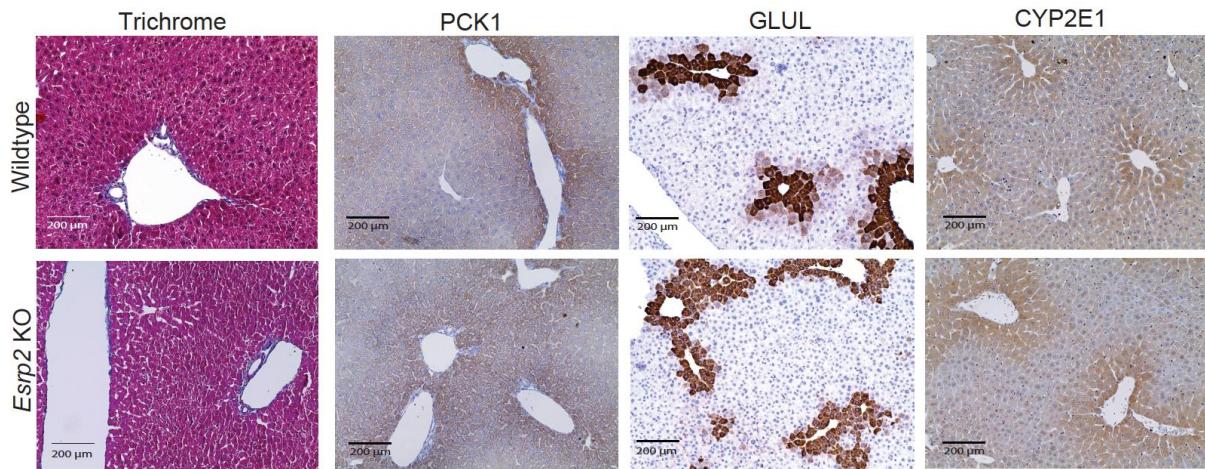
Supplementary Figure 5.



Supplementary Figure 5. Phenotypic characterization of *Esrp2* KO mouse

(a) Increased number of phospho-histone 3 (pHIST3H3) positive cells in *Esrp2* KO livers (green arrows), Scale bars, 200 μ m; quantification of Ki-67 and pHIST3H3 positive cells. mean \pm SD **(b)** No significant difference in apoptosis between WT and *Esrp2* KO animals as shown by TUNEL assay, Scale bars, 100 μ m. White arrows point to apoptotic nuclei. Bar graph indicates the quantification of average number of apoptotic cells per field in WT and *Esrp2* KO sections. mean \pm SD **(c)** Blood serum levels of ALT, AST, cholesterol, triglycerides, random and fasting glucose levels of WT and *Esrp2* KO mice. mean \pm SD , P<0.005, Student's t-test

Supplementary Figure 6.

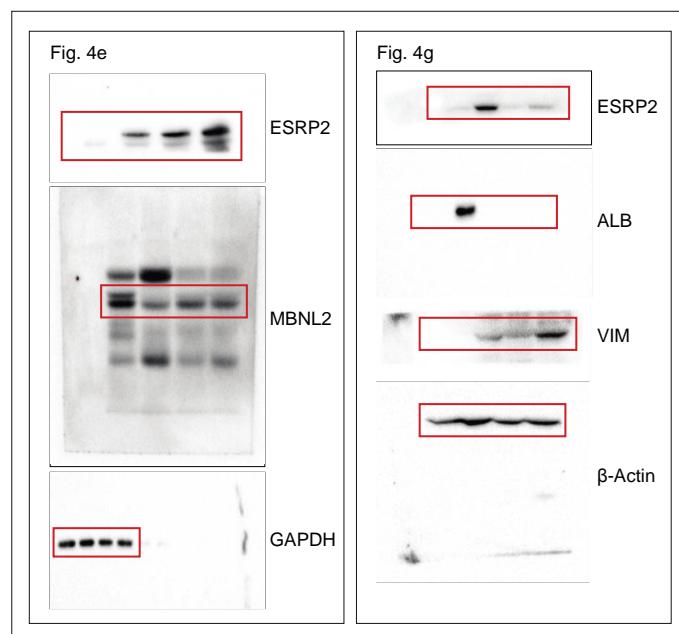


Supplementary Figure 6. Characterization of liver zonation in *Esrp2* KO mice

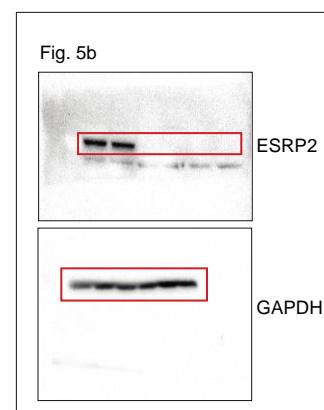
No apparent fibrosis in WT or *Esrp2* KO animals as evidenced by trichrome staining; reduced and diffused periportal marker phosphoenolpyruvate carboxykinase (PCK1) staining in *Esrp2* KO compared to WT; no difference in perivenous staining of glutamine synthetase (GLUL); slightly diffused cytochrome P450 2E1 (CYP2E1) staining in perivenous region in *Esrp2* KO compared to WT. Scale bars, 200 μ m

Supplementary Figure 7.

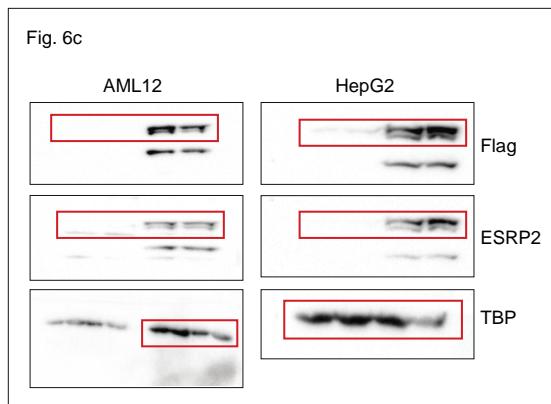
a



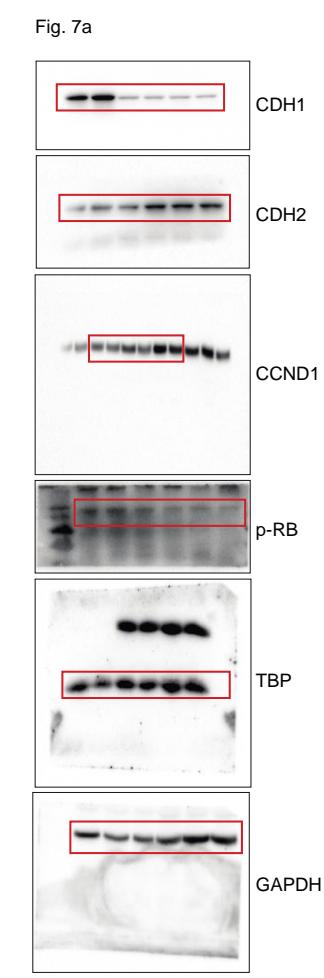
b



c



d



Supplementary Figure 7. Uncropped images of immunoblots shown in Fig. 4 to Fig. 7

Uncropped images of immunoblots displayed in **(a)** Fig. 4e and f; **(b)** Fig. 5b; **(c)** Fig. 6c; **(d)** Fig. 7a are presented here. Red boxes indicate the lanes used in the Figures.

Supplementary Table 1: RNA-Seq Analysis

Sample Name	Read length	Total reads	Uniquely mapped reads	Mapping rate
Liver embryonic day 18 rep1	101x2	3,718,224	3,255,332	87.5%
Liver embryonic day 18 rep2	101x2	3,555,666	3,136,002	88.2%
Liver postnatal day 14 rep1	101x2	3,220,400	2,856,919	88.7%
Liver postnatal day 14 rep2	101x2	3,795,466	3,313,816	87.3%
Liver postnatal day 28 rep1	101x2	3,618,022	3,131,980	86.5%
Liver postnatal day 28 rep2	101x2	3,569,840	3,147,281	88.1%
Liver Adult rep1	101x2	3,616,912	3,207, 804	88.6%
Liver Adult rep2	101x2	3,640,783	3,233,538	88.8%

Supplementary Table 2: Primer Sequences

Gene name	Primer Sequence (5'-3')		Amplicon Size
	Forward	Reverse	
Mouse Primers for qRT-PCR assays			
Celf1	CAGATTGAAGAGTGCCGGATA	TAGCTGTCTGTGCCATGGTT	97
Celf2	ACTTGGGGGAAACCTAACAGG	CTGAATGCCACTGAATGCAC	105
Esrp2	TATAAAGCCACAGGGGAGGA	TCTTCCC GTGATAGGAAACG	79
Hnrnp A	ATTTGGTCGAGGAGGGAAC	ATTATAGCCATCCCCACTGC	92
Hnrnp H	GCAGAGGAGCTGGTTTGAG	GAACCAAATCCATAGCCATCA	102
Hnrnp L	CTGCTTGTATGGCAATGTGG	AGCATAGCCATCAGCCATT	85
Hnrnp LL	CTGGCTCCGTTGAATGGTT	GTGCCAGGAATGGTCTTCAT	124
Mbnl1	GGAGTTCAGTGCCAGCAG	CACGCTGGTACTCTCGACAC	88
Mbnl2	GGCTCACTGCAACTCAGAA	AGCGGCAGTCTGTCTCTCC	100
Ptbp1	CGTTCACCAAGAACAAACCAG	TTGTAGATGTTCTGGCCATCC	100
Srsf1	CGCTTAGACCTTCCTACTGGTG	CCCTGCATATGGAGAGGACA	99
Srsf2	CCACCCC GTCGGTACG	CGACCTGGACCGACTCC	99
Cyp2b10	TGCTGTCGTTGAGCCAACC	CCACTAAACATTGGGCTTCCT	161
Albumin	TGCTGAGACTTGCCAAGACA	TCCATATTCTCCAAGCTTCTCGT	170
Cyp3a11	TCTCATAAAGCCCTTCTGA	AATGCAGGGTGAAGGAAAGT	103
Fbp1	TGAGCCTCTGCGAAGGATG	GAAGCAGTTGACACCAAT	118
Igfr2	AGAGAGGAAGGAGACAACTG	CAAGTAGTAGGTGTACTCGC	117
Vimentin	ACCAAGGTCTGTGCCTCGTC	AATAGAGGCTCGGGTAGTG	154
Meg3	TCCTCACCTCCAATTCCCC	GAGCGAGAGCCGTTCGATG	71
Cyclin E	GATCGTTACATGGCATCACA	AAACTGGTGCAACTTGGAG	120
Cyclin B2	GCCAAGAGCCATGTGACTATC	CAGAGCTGGTACTTGGTGTTC	114
E2f8	GCCTCTT CCTGCCTCCTAG	GGAGCGGA ACTGATCTTCCT	159
p27	CAGAATCATAAGCCCCCTGGA	GGTCCTCAGAGTTGCCTGA	190
Cdk1	GCCAGATAGTGGCCATGAAG	TCCATGGACAGGA ACTCAA	178
Cdk6	AATCTGCTCAACCCATCGAG	GTTGGATGGCAGGTGAGAGT	186
Aqp7	AAGTGTTCAGAGCCGGAAAC	GGGTGAATTAAACCCAGGTA	100
Gapdh	AACGACCCCTTCATTGAC	TCCACGACATACTCAGCAC	191
Beta-Actin	CCCTAAGGCCAACCGTGAAA	CGGAGTCCATCACAATGCCT	134
Human Primers for qRT-PCR assays			
Gene name	Primer Sequence (5'-3')		Amplicon Size
	Forward	Reverse	
CELF1	CAGACGGCTATCAAGGCAAT	TGGGCCATTCTCTTCTGTT	113
CELF2	CACCAATGCAAACCCCTCT	CGAGAGAGGTCAAGGAGTTCA	119
ESRP2	CCCTACATGCTCTGCACTGA	GGAATTCTCTCGGAGGTCA	124
HnRNP A	AGGCAGTGGCAAGAAAAGG	CAGTTGTGGCCATT CACAGT	105

HnRNP H	AGCTGGCTTGAGAGGATGA	TCTGACCCAAATCCATAGCC	99
HnRNP L	CTGGGGACTCGGATGACTC	ACAGGGCCACAAGGATTACA	118
HnRNP LL	GGAGGGGGAGATCGACTACT	ACGACGGGTGAAAACAGAAC	150
MBNL1	CATTGCAAGCCAAGATCAA	AGCAGGCCTCTTGGTAATG	129
MBNL2	GCCCAGCAGATGCAATTAT	GGAGCAAAGCTAATAGCCGTA	108
PTBP1	GTTCGGCACAGTGTGAAGA	CAGCAGGCGTTGTAGATGTT	135
SRSF1	GCGACATCGACCTCAAGAAT	GCAGACGGTACCCATCGTAA	126
SRSF2	GTCGACCTCCAAGTCCAGAT	TTGGATTCCCTCTTGGACAC	129
Beta-Actin	CTGGAACGGTGAAGGTGACA	AAGGGACTTCCTGTAACAATGCA	145
GAPDH	CGAGATCCCTCCAAAATCAA	GGCAGAGATGATGACCCTT	132

TaqMan Probes from Life Technologies

Gene name	Catalogue Number	Assay ID	Amplicon Size
ESRP1	4351372	Mm01220936_g1	97
ESRP2	4331182	Mm00616290_m1	68

Primers for adenovirus cloning

Gene name	Forward	Reverse	
ESRP2	gtaactataacggtcATGGATTACA AGGATGACGATGACAAGACTC CGCCGCCGCCG	attacctttctccCTACAAACACACCCACTCCTTAGG GGCTTG	

Supplementary reference:

1. Uhlen, M. et al. Proteomics. Tissue-based map of the human proteome. *Science* **347**, 1260419 (2015).